Fulminant hepatic failure resulting from coexistent Wilson's disease and hepatitis E

R Sallie, J Chiyende, K C Tan, D Bradley, B Portmann, Roger Williams, A P Mowat, G Mieli-Vergani

Abstract

Fulminant hepatic failure resulting from hepatitis E and coexistent Wilson's disease was diagnosed in a six year old girl six weeks after returning from a holiday in India. Wilson's disease was diagnosed on the basis of histological evidence of hepatocellular copper deposition, confirmed by biochemical estimation of liver copper concentration. Although severely damaged, the liver was non-cirrhotic. Hepatitis E virus (HEV) was diagnosed by nested polymerase chain reaction, the specificity of which was confirmed by direct sequencing of amplified DNA. Replication of HEV within the liver at the time of diagnosis was confirmed by selective amplification of the antigenomic strand of the virus obtained from total liver RNA. The patient had an orthotopic liver transplantation without recurrence of hepatitis and remains well at 19 months. Viral excretion, recorded by serial amplifiation of HEV RNA extracted from stool samples, persisted for 30 days after liver grafting. Severe vitiligo, present preoperatively, dramatically improved after liver grafting and institution of immunosuppressive treatment. This case suggests that viral infection may play a part in the acute decompensation seen in some cases of Wilson's disease.

(*Gut* 1994; 35: 849–853)

Fulminant Wilson's disease is characterised by severe derangement of liver function, encephalopathy, and haemolysis in patients with previously undiagnosed liver disease. Early diagnosis is essential as without orthoptic liver transplantation, the mortality is virtually 100%. Although the basic defect, a failure of biliary copper excretion, has been known for many years, the exact pathophysiology of the defective excretory mechanism has remained elusive. It is known that Wilson's disease may run a varied course, ranging from fulminant Wilson's disease to an insidious progression to cirrhosis over 20–30 years. The factors that dictate disease tempo in any particular patient remain unknown. Many patients with fulminant Wilson's disease have grossly raised serum copper concentrations, and it has been postulated that an acute insult damages the hepatocytes, which release free copper in toxic concentrations, which in turn damage cell membranes both directly[1] and by free radical mediated damage.[2] It is therefore possible that in some patients a hepatic injury unrelated to the metabolic abnormalities of Wilson's disease in itself may be the primary process that triggers fulminant Wilson's disease. This study reports on a case of fulminant hepatic failure occurring in a six year old girl with biochemical and histopathological evidence for underlying Wilson's disease complicated by hepatitis E virus (HEV) infection. The diagnosis of HEV was made by nested polymerase chain reaction and confirmatory DNA sequencing. The patient required emergency orthotopic liver transplantation. The histopathology of HEV, the time course of viral shedding in stool, the viral titre in serum, and the relation to Wilson's disease are discussed.

Institute of Liver Studies
R Sallie
B Portmann
Roger Williams

Department of Child Health
J Chiyende
G Mieli-Vergani
A P Mowat

and Department of Surgery
K C Tan

King's College Hospital, Denmark Hill, London

Hepatitis Branch, Centres for Disease Control, Atlanta, Georgia, USA
D Bradley

Correspondence to:
Dr G Mieli-Vergani, Department of Child Health, King's College Hospital, Denmark Hill, London SE5 8RX.

Accepted for publication 20 September 1993

Case report

A six year old girl, born and resident in Birmingham, England to unrelated Indian parents was well except for severe vitiligo (Fig 1A), which started at four and a half years of age. Twelve weeks before presentation, the patient left for a six week holiday to Bombay, India. Before leaving the United Kingdom she was given anti-typhoid and anti-cholera vaccinations and normal immunoglobulin prophylaxis. She was well and without symptoms while in India. Two weeks after her return to the UK, she developed diarrhoea, and a flu like illness with arthralgia and a non-pruritic rash. Other family members were asymptomatic. Severe jaundice developed and the patient was referred three weeks after the onset of symptoms. Dietary copper intake was normal and there was no history of copper intoxication. On physical examination the liver was of normal size and the spleen impalpable. She was not encephalopathic. Serum biochemistry on admission suggested severely deranged liver function (bilirubin 550 μmol/l, normal (n) <20 <μmol/l; aspartate aminotransferase 640 IU/l, n<50 IU/l; alkaline phosphatase 1081 IU/l, n<350 IU/l; albumin 29 g/l, n=35–45 g/l). International normalised ratio (INR) increased from 2·9 to 4·9 in the first 12 hours after admission. Haematological investigations were compatible with Coombs' negative haemolytic anaemia: Hb 7·9 (n=13·4–16·5 gm/dl), reticulocyte count 220×10^9/l (n=50–100×10^9), white cell count $9{\cdot}4\times10^9$/l (n=4–11×10^9), platelets 220×10^9/l (n=200–400×10^9). Serological tests for hepatitis B, hepatitis A, Ebstein-Barr virus, human immunodeficiency virus, herpes viruses, and leptospira were negative while cytomegalovirus titre (1:16) was unremarkable. There were no serological features of autoimmune hepatitis (anti-smooth muscle antibody, anti-nuclear antibody, anti-liver/kidney microsomal antibody, and anti-gastroparietal cell antibody negative) and serum immunoglobulins were normal. Anti-liver specific lipoprotein was positive at a titre of 1:1050 and anti-asialoglycoprotein receptor was also

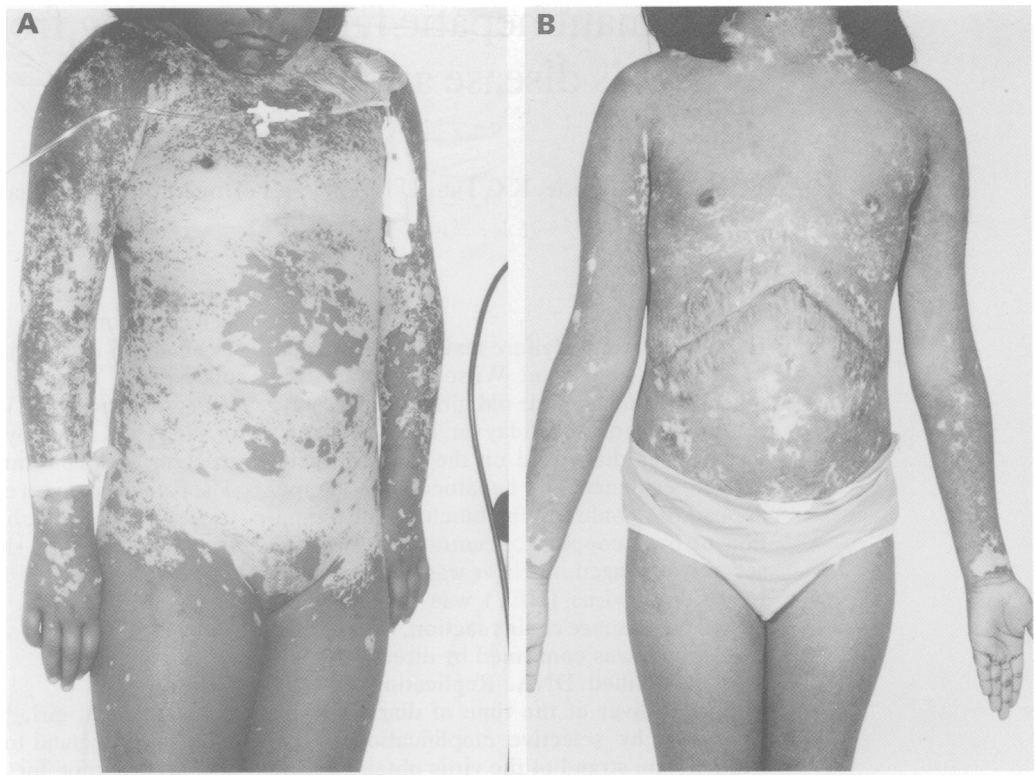

Figure 1: (A) Frontal trunk view of the patient immediately before orthotopic liver transplantation. The anterior part of the trunk was most widely affected by vitiligo but face and back were also severely depigmented in parts; (B) frontal view taken six months after transplantation. Note depigmentation much less severe in intensity and less extensive in distribution.

positive at a titre of 1:50. Slit lamp examination for Kayser-Fleischer rings was negative. During the patient's stay in hospital, liver function progressively deteriorated, she became encephalopathic with asterixis and confusion and worsening coagulopathy (maximum INR 8·3 despite replacement treatment). Copper studies were consistent with the diagnosis of Wilson's disease,[3] and a decision to undertake orthotopic liver transplantation was taken 10 days after admission.

Methods

At the time of liver transplantation, serum, the resected liver, and bile obtained at direct puncture of the removed gall bladder were examined for hepatitis C and E virus nucleic acid by nested polymerase chain reaction (PCR). Ribonucleic acid was extracted from serum, bile, and liver by solubilisation 50:50 in 8 molar guanidinium isothiocyanate as described by Chomczynski *et al*,[4] before phenol/chloroform extraction and overnight ethanol precipitation. Complementary DNA (cDNA) was synthesised using avian myeloblastosis virus (AMV, Promega, UK) mediated reverse transcription using primer RS HEV2 5'-TCTACTTCAACTTCAAGCC-3' to initiate the reverse transcription reaction. Polymerase chain reaction was carried out on the cDNA thus synthesised using RS HEV1 5'-ACAGGTGAGACCATTGCC -3' as its primer pair, and cycling parameters of 95 degrees for one minute, 55 degrees for one minute, and 72 degrees for one minute for 25 cycles. These primers theoretically produce an amplicon of about 573 pairs in size. Five µl of the resultant first round reaction were then used as a template for a second round PCR using RS HEV3 5'-TTGATGACACCGTCTTCTGGC-3' and RS HEV4 5'-CAGTATTCCATAGAAGAGTGCC -3', which produce an amplicon of 266 base pairs when HEV is correctly amplified. Albumin mRNA was amplified as an internal control. Appropriate positive and negative controls for PCR amplification were used for all experiments, which were performed in triplicate. Hepatitis C virus (HCV) was also sought by nested PCR using the primers described by Ulrich *et al*.[5] To examine the temporal changes in viral excretion serum samples were obtained at least thrice weekly, cDNA was made and serially 10-fold diluted to obtain a viral PCR titre, as described by Simmonds[6] and Fong[7] for quantification of HCV. Stools were collected and an aliquot mixed 1:20 in guanidinium isothiocyanate before RNA extraction, reverse transcription, and PCR. To evaluate viral replication, antigenomic (negative strand) RNA was selectively primed and amplified in an analogous manner to that applied to the investigation of the replicative intermediates of HCV.[7]

Methods to determine urine, serum, and liver tissue copper concentrations and caeruloplasmin concentration were those previously described.[3]

Results

Figure 2 shows the initial PCR findings in liver, serum, and bile and show a positive nested PCR signal for HEV in the patient's serum, bile, and liver tissue. Although a distinct band at the appropriate molecular weight was obtained, the specificity of this product was confirmed by direct, bidirectional sequencing of the PCR product by Sanger chain termination sequencing

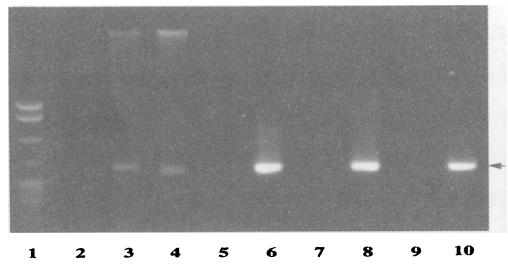

Figure 2: Ethidium bromide stained 3% NuSieve 1% agarose gel electrophoresis of polymerase chain reaction products. Lane 1: molecular weight standard (Msp digested f 174 DNA); lane 2: negative reagent control; lane 3: albumin mRNA amplicon 277 base pairs (internal positive control for liver amplification); lane 4: positive control for HEV (ETl·1 clone, 266 base pairs); lane 5: normal human serum; lane 6: patient's serum; lane 7: normal human bile; lane 8: patient's gall bladder bile; lane 9: normal human liver; lane 10: patient's liver.

chemistry using the Femptomole (sequencing kit; Promega, UK) (data not shown). Both positive and negative stranded HEV RNA was detected in liver tissue at transplantation, indicating active viral replication at the time of grafting (Fig 3). Selective PCR of both strands, however, from the two subsequent biopsy specimens (performed to investigate deteriorating liver function) showed only the presence of positive stranded forms. Serial examinations of serum and stools by nested PCR confirmed continued viraemia and excretion of HEV in stools for four weeks after orthotopic liver transplantation, while viral titres, as defined by serial end point dilution of the cDNA synthesised from undiluted serum, showed an early biphasic fluctuation. No evidence of HCV infection was found.

Biochemical estimation of tissue copper was consistent with homozygous Wilson's disease and showed massively raised copper stores with substantial regional variation of copper concentration within the resected liver (134, 403, 536, 707 μg/g dry weight, mean 445 μg/g dry weight, n<50 μg/g dry weight), with areas of regeneration being least overloaded. Serum caeruloplasmin was low (0·09 g/l, n 0·2–0·6 g/l), total serum copper was normal (12·0 μmol/l, n 11–20 μmol/l), free serum copper was not measured. Urinary 24 hour copper estimation was also grossly raised (15 μmol/24 hours, n<1·25 μmol/24 hours) while urinary copper excretion post D-penicillamine challenge (500 mg at a 12 hour interval orally) was also increased (28·7 μmol/24 hours) to a value typical of Wilson's disease.[3 8] Investigation of the patient's immediate family (both parents and a younger brother) was uninformative as serum copper, caeruloplasmin, and urinary copper excretion were normal in all.

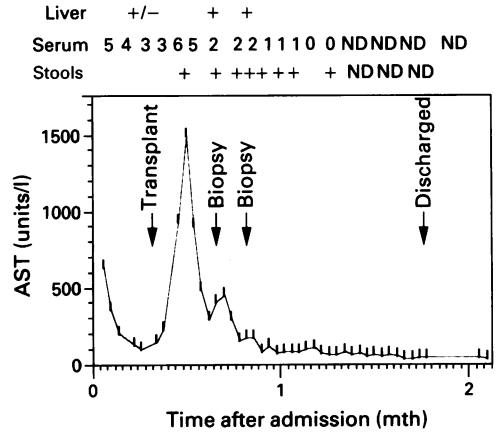

Figure 3: Time course of viral carriage and excretion compared with clinical events and serum aspartate aminotransferase (AST). Polymerase chain reaction (PCR) results expressed as follows: liver = +/− denotes detection of both positive and negative strand, + denotes positive strand alone; serum = numbers refer to the highest log dilution of cDNA at which the ethidium bromide stained PCR signal was visible; stool = + denotes detection of HEV by PCR. ND = not detected. Note two episodes of deterioration in AST because of acute rejection, which were not associated with detection of negative strand RNA within the liver tissue.

HISTOPATHOLOGY

Macroscopically, the excised liver was small (weight = 310 g) with broad areas of multiacinar collapse and scattered foci of nodular and cholestatic parenchyma. Histological examination (Fig 4) showed that in the collapsed areas there was extensive cell loss and mixed inflammatory cell infiltrate, with periportal ductular structures and evidence of hepatic venulitis (Fig 4A, B). In less affected areas the parenchyma showed features of regeneration with large cholangiolar bile casts and areas of fatty infiltration (Fig 4C). Prominent areas in periportal distribution showed hepatocyte ballooning and cytoplasmic vesiculation with single cell necrosis and shrunken, irregular shaped eosinophilic cells. Orcein staining showed random deposits of fine copper associated protein granules, which gave positive, yet weaker staining with rhodamine (Fig 4D). There was no evidence of cirrhotic transformation, but pre-existing fibrosis could not be excluded because of the overall derangement of the liver architecture.

CLINICAL OUTCOME

After orthotopic liver transplantation, performed with a 'cut down' left lobe graft, the patient required a further laparotomy for intra-abdominal bleeding six hours later. In the next 24 hours, the coagulopathy improved and the bleeding settled the day after liver grafting. Abdominal sepsis and intestinal obstruction required two further laparotomies and drainage of subhepatic collections at 12 and 35 days respectively. After grafting, aspartate aminotransferase activity was initially grossly raised, reflecting preservation injury and reductive surgery but apart from two episodes of mild acute rejection, liver function tests progressively returned towards normal (Fig 3). The patient was discharged from hospital after two months and by six months both the extent and the severity of the vitiligo had dramatically improved (Fig 1B). At 19 months after initial presentation the patient remains well, with normal graft function on a maintenance immunosuppressive regimen of cyclosporin 170 mg/day (4 mg/g body weight), azathioprine 25 mg/day, and prednisolone 3 mg/day.

Discussion

The clinical presentation of this patient is

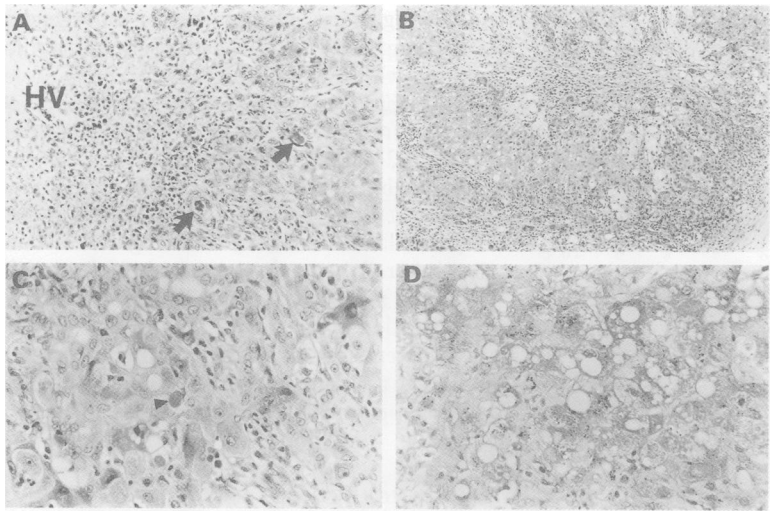

Figure 4: (A) Liver histology (haematoxylin and eosin, original magnification ×150) showing broad areas of parenchymal collapse with mild hepatic venulitis (HV). Note the presence of cholangiolar bile casts at the interface between areas of collapse and regenerative parenchyma; (B) liver section showing bridging collapse, aggressive inflammation and ballooning hepatocytes (haematoxylin and eosin, original magnification ×75); (C) less affected parenchyma showing ongoing damage with mild fatty change and single cell drop out with acidophilic bodies and shrunken, irregularly shaped hepatocytes (haematoxylin and eosin ×300); (D) similar area to (C) showing parenchymal deposits of copper associated protein granules (orcein original magnification ×300).

unusual, firstly because she is the youngest patient with fulminant Wilson's disease seen in our unit[8]; secondly, because, in noticeable contrast with previously reported cases of fulminant Wilson's disease,[9] she did not have clear histological evidence of either underlying cirrhosis or chronic liver damage. It is probable that the sudden deterioration of her liver function leading to hepatic failure was brought about by the superimposed acute hepatitis E. Fulminant hepatic failure resulting from hepatitis E, well recognised during pregnancy[10] and in neonates[11] is, however, distinctly rare in children. It is possible that the severity of the liver damage in this case derives from the coincidental presence of two rare conditions. The acute viral insult superimposed to the underlying Wilson's disease may have caused release of free copper in sufficient concentrations to provoke haemolysis and progressively more severe liver damage. It is also possible that the pre-existing Wilson's disease may have predisposed the girl, if not to viral infection, to an exacerbation of its consequences. It is conceivable that viral infections, particularly those producing hepatitis, may be responsible, at least in some cases, for the acute decompensation seen in fulminant Wilson's disease.

Diagnosis of Wilson's disease in the context of fulminant hepatic failure is notoriously difficult because abnormal serum copper concentration, high urinary copper excretion, and low caeruloplasmin concentrations are common in acute liver failure not caused by Wilson's disease.[3] Typically, though, these patients have low liver copper content, because of severe liver cell necrosis.[3] In the absence of chronic cholestatic liver disease or high oral copper intake, abnormal biochemical copper studies in conjunction with histopathological evidence of excess copper and copper associated protein in a non-cirrhotic liver are diagnostic of homozygous Wilson's disease in its early stages. In our case, the 24 hour urinary copper excretions before and after penicillamine challenge were at the values seen in Wilson's disease[3] and the liver copper content was 3–15 times the upper limit of normal, well within the range seen in fulminant Wilson's disease.[8] Because of the ethnic origin of our patient, Indian childhood cirrhosis, which is also characterised by grossly raised tissue copper concentrations,[12] should be considered. Indian childhood cirrhosis, however, is exceptionally rare outside India,[13] generally presents earlier in life with an insidious cirrhosis, rarely if ever produces fulminant hepatic failure,[12] and has both a characteristic histological pattern of coarse granular deposits of copper associated protein,[14] not seen in our patient, and a normal serum caeruloplasmin.[15]

As recently described in the course of acute hepatitis E transmitted to a volunteer,[16] our patient had detectable HEV RNA both in serum and stools at the time of acute symptomatic hepatitis. She continued to be serum and stool HEV RNA positive for about one month after transplant. As Figure 3 shows, viral titres showed a biphasic pattern early in the patient's hospital course, probably reflecting the severity of hepatic necrosis and release of virions into the serum rather than a true variation in the rate of viral replication. Antigenomic or negative stranded HEV RNA was detected in the resected liver, confirming the presence of active viral replication, whereas attempted selective amplification of both genomic and antigenomic RNA from two subsequent biopsy specimens showed only the presence of genomic strands, suggesting the cessation of active viral replication, or the amplification of viral RNA in blood trapped within the liver biopsy specimen.

The initial early rise in serum transaminases seen after transplantation in this patient provoked concern about recurrence of hepatitis, but the biopsy features were those of mild cellular rejection, and no clinical evidence of recurrent hepatitis was seen. We do not know why hepatits E did not recur. The clinical course of hepatitis E, however, is similar to that of hepatis A, the recurrence of which after transplant is exceptional.[17]

The epidemiological evidence of faecal-oral transmission of HEV,[18] in addition to the finding of viral like particles in stools[10 16] and bile ducts,[19] and its cloning from the bile of experimentally infected cyanomologus macaques[20] show that viral particles are excreted in bile or by biliary cells. In the few cases of fulminant hepatitis E with well recorded pathology in English language medical publications,[19] cholestasis seems a prominent feature of the disease. It is conceivable that the excretory defect in Wilson's disease may result in defective viral excretion with failure to clear the virus and consequent particularly severe hepatocellular damage. If this hypothesis is correct, the oestrogen induced bile secretion impairment seen in some women during pregnancy and the comparatively immature bile secretory apparatus in neonates may explain the high death rate for HEV seen in these groups of patients.

Finally, although the severe vitiligo of this case made us suspect an autoimmune pathogenesis for

her liver disease, she did not have non-organ specific autoantibodies or increased immunoglobulin G, typically present in autoimmune hepatitis. The increased titre of anti-liver specific lipoprotein and the low titre of anti-asialoglycoprotein protein antibodies are compatible with severe Wilson's disease (unpublished data). The dramatic improvement of the vitiligo after immunosuppressive treatment/liver grafting was unexpected. Before orthotopic liver transplantation the patient's vitiligo had been a disfiguring social handicap. By six months after transplantation, however, both the extent and the severity of depigmentation had greatly improved. While we would not advocate immunosuppressive treatment for vitiligo, the clinical response in this patient was certainly impressive and emphasises the probable autoimmune pathogenesis of this condition.

1 Aaseth J, Ribarov S, Bochev P. The interaction of copper (Cu++) with the erythrocyte membrane and 2,3-dimercaptopropanesulphonate in vitro: a source of activated oxygen species. *Pharmacol Toxicocol* 1987; **61**: 250–3.

2 Fernandes A, Mira ML, Azevedo MS, Manso C. Mechanisms of hemolysis induced by copper. *Free Radic Res Commun* 1988; **4**: 291–8.

3 Martins da Costa C, Baldwin D, Portmann B, Lolin Y, Mowat AP, Mieli-Vergani G. Value of urinary copper excretion after penicillamine challenge in the diagnosis of Wilson's disease. *Hepatology* 1992; **15**: 609–15.

4 Chomczynski P, Sacchi N. Single-step method of RNA isolation by acid guanidinium thiocyanate-phenol-chloroform extraction. *Anal Biochem* 1987; **162**: 156–9.

5 Ulrich PP, Romeo JM, Lane PK, Kelly I, Daniel JJ, Vyas GN. Detection, semiquantitation, and genetic variation in hepatitis C virus sequences amplified from the plasma of blood donors with elevated alanine aminotransferase. *J Clin Invest* 1990; **86**: 1609–14.

6 Simmonds P, Zhang LQ, Watson HG, Rebus S, Ferguson ED, Balfe P, *et al.* Hepatitis C quantification and sequencing in blood products, haemophiliacs, and drug users. *Lancet* 1990; **336**: 1469–72.

7 Fong TL, Shindo M, Feinstone SM, Hoofnagle JH, DiBisceglie AM. Detection of replicative intermediates of Hepatitis 'C' viral RNA in liver and serum of patients with chronic hepatitis C. *J Clin Invest* 1991; **88**: 1058–60.

8 Sallie R, Katsiyannakis L, Baldwin D, Davies S, O'Grady J, Mowat AP, *et al.* Failure of simple biochemical indices to differentiate fulminant Wilson's disease from other causes of fulminant liver failure. *Hepatology* 1992; **16**: 1206–11.

9 Davies SE, Williams R, Portmann B. Hepatic morphology and histochemistry of Wilson's disease presenting as fulminant hepatic failure: a study of 11 cases. *Histopathology* 1989; **15**: 385–94.

10 Bradley DW. Enterically-transmitted non-A, non-B hepatitis. *Br Med Bull* 1990; **46**: 442–61.

11 Song DY, Zhuang H, Kang XC, Liu XM, Li Z, Hao W, *et al.* Hepatitis E in Hetian City: a report of 562 cases. In: Hollinger FB, Lemon SM, Margolis HS, eds. *Viral hepatitis and liver disease.* Baltimore: Williams and Wilkins, 1990: 528–9.

12 Bhave SA, Pandit AN, Pradhan AM, Sidhaye DG, Kantarjian A, Williams A, *et al.* Liver disease in India. *Arch Dis Child* 1982; **57**: 922–8.

13 Weiss M, Muller HJ, Wiebecke B, Belohradsky BH. First description of 'Indian childhood cirrhosis' in a non-Indian infant in Europe. *Acta Paed Scand* 1989; **78**: 152–6.

14 Portmann B, Mowat AP, Tanner MS, Williams R. Orcein-positive liver deposits in Indian childhood cirrhosis. *Lancet* 1978; **i**: 1338–40.

15 Barrow L, Tanner MS. Copper distribution among serum proteins in paediatric liver disorders and malignancies. *Eur J Clin Invest* 1988; **18**: 555–60.

16 Chauhan A, Jameel S, Dilawari JB, Chawla YK, Kaur U, Ganguly NK. Hepatitis E virus transmission to a volunteer. *Lancet* 1993; **341**: 149–50.

17 Fagan EA, Yousef G, Brahm J, Mann G, Wolstenholme A, Portmann B, *et al.* Persistence of hepatitis A virus in fulminant hepatitis and after liver transplantation. *J Med Virol* 1990; **30**: 131–6.

18 Khuroo MS. Study of epidemic of a non-A non-B hepatitis: possibility of another human hepatitis virus distinct from post-transfusion non-A non-B type. *Am J Med* 1980; **68**: 818–24.

19 Asher LV, Innis BL, Shrestha MP, Ticehurst J, Baze WB. Virus-like particles in the liver of a patient with fulminant hepatitis and antibody to hepatitis E virus. *J Med Virol* 1990; **31**: 229–33.

20 Reyes GR, Purdy MA, Kim JP, Luk KG, Young LM, Fry KE, *et al.* Isolation of a cDNA from the virus responsible for enterically transmitted non-A, non-B hepatitis. *Science* 1990; **247**: 1335–9.